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CARDIOVASCULAR GENOMIC MEDICINE

Redefining Risk in Acute Coronary Syndromes Using Molecular Medicine

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Acute coronary syndromes represent a complex phenotype involving the interplay of many elements. The risk of developing an acute coronary syndrome and related complications has been defined by variables such as age, diabetes, smoking history, serum creatine phosphokinase, or electrocardiographic findings. However, in the past 5 years the wide-scale acceptance of a protein—troponin—has changed the diagnostic profile. With advances in molecular medicine, this protein is a segue to a panel of molecular assays that will improve screening and tailored intervention. We expound upon some of these factors and the potential they may carry in changing clinical medicine. (J Am Coll Cardiol 2007;49:279–89) © 2007 by the American College of Cardiology Foundation

Coronary artery disease (CAD) is increasing in prevalence and is predicted to become the dominant cause of mortality worldwide by 2020. A burgeoning body of literature exists that implicates inflammation as being central to atherogenesis and, ultimately, atherothrombosis (1). In fact, the inflammatory process appears to be more extensive than previously thought and may involve multiple vulnerable plaques within the coronary bed and, in many patients, other arterial trees simultaneously (2). That an integral link among inflammation, atherogenesis, and atherothrombosis exists is fundamental to understanding acute coronary syndromes (ACS). Translating this understanding and the emerging concept of differential genetic heritability between myocardial infarction (MI) and atherosclerosis (3) into the development of quantifiable molecular risk factors in otherwise healthy, asymptomatic individuals is a major goal for prevention.

As the mechanisms and pathways involved in the processes of plaque rupture, thrombosis, and response to injury are defined, a logical evolution would be to use this opportunity to better define risk of future events and complications. Use of molecular markers of inflammation after ACS to predict the likelihood of recurrence or even appropriate response to therapy may facilitate targeted therapeutic strategies based on a comprehensive molecular risk profile (4) rather than on demographic and clinical characteristics.

The goal of this review is to provide insight into the complex interactions between the inflammatory and cellular mechanisms involved in the pathogenesis of ACS and the response to injury. The prognostic value of some of these novel markers and relevant data on proposed therapeutic interventions will be addressed so that in time we can use these markers to prevent ACS events or, at least improve, clinical outcomes.

Endothelium

Compromise of endothelial integrity is felt to be fundamental, not only to the initiation and progression of atherosclerotic disease, but also to the onset of ACS. Leukocytes are believed to contribute to direct endothelial damage in this setting. Irrespective of the underlying contributor, endothelial damage and dysfunction remain integral to atherogenesis and the development of an ACS.

Circulating endothelial cells as a marker of panvascular injury. Circulating endothelial cells are a marker of arterial injury in vascular disease. Notably, significantly elevated levels of circulating endothelial cells have been observed in patients with ACS compared with those with stable angina (5). More recently, it was discovered that elevated levels of circulating endothelial cells measured in patients within 48 h of an ACS independently predict subsequent short- and long-term outcomes (6).

Role of the subendothelial matrix von Willebrand factor (vWF) in ACS. Through the actions of a key component, vWF, on factor VIII activity, the matrix contributes to modulation of the coagulation cascade and to the pathogenesis of ACS. Beyond its role in facilitating coagulation protein interaction, vWF binds to subendothelial collagen via its A3 domain and initiates platelet adhesion via the glycoprotein (GP) 1b receptor (7). Experimental evidence suggests that both vWF and high shear stress may be responsible for platelet aggregation in acute MI. Conversely, inhibition of the GP1b receptor from serum of patients with an acute MI or unstable angina by a vWF antibody results in reduced shear-induced platelet aggregation (8). Ultimately, increased levels of vWF

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Abbreviations and Acronyms

ACS = acute coronary syndrome
CAD = coronary artery disease
CRP = C-reactive protein
EPC = endothelial progenitor cells
GP = glycoprotein
FLAP = 5-lipoxygenase activating protein pathway
LDL = low-density lipoprotein
MI = myocardial infarction

have been associated with suboptimal angiographic results and increased adverse events across the spectrum of ACS (9,10).

Platelets

The importance of platelets in thrombosis and ACS is well established. Through release of various constituents, expression of various receptors, and interactions with leukocytes and the endothelium, platelets function as inflammatory mediators in patients with ACS (Fig. 1). Platelets provide a pivotal link between inflammation and thrombosis in ACS.

CD40 and CD40L. CD40 and CD40L have been found on platelets and several other cell types in functional-bound and soluble (sCD40L) forms. Although many platelet-derived factors have been identified, recent evidence suggests that CD40L is actively involved in the pathogenesis of ACS. Through direct platelet-to-cell stimulation, most notably the interaction between CD40L on activated platelets and the CD40 receptor on endothelial cells, CD40L, drives the inflammatory response. Such interactions facilitate increased expression of adhesion molecules on the surface of endothelial cells and release of various stimulatory chemokines. These events, in turn, facilitate activation of circulating monocytes as a trigger of atherosclerosis (11).

Both CD40L and sCD40L contain separate domains allowing for direct binding to the $\alpha_{IIb}B_3$-receptor on platelets. It has been suggested that this CD40L-platelet $\alpha_{IIb}B_3$ receptor interaction is important for stability of platelet-based thrombus (12). Stimulation of the $\alpha_{IIb}B_3$ receptor is known to release sCD40L from within platelets (13) in addition to activating other platelets.

Beyond known proinflammatory and thrombotic properties of CD40L, experimental evidence suggests that CD40L-induced platelet activation leads to the production of reactive oxygen and nitrogen species, which are able to prevent endothelial cell migration and angiogenesis (14). As a consequence of inhibiting endothelial cell recovery, the risk of subsequent coronary events may be greater.

Clinical studies have supported the involvement of CD40L in ACS and the prognostic value in ACS populations. Levels of sCD40L have been shown to be an independent predictor of adverse cardiovascular events after ACS (15) with increased levels portending a worse prognosis (16,17). Importantly, specific therapeutic strategies have shown to be beneficial in reducing risk associated with sCD40L (Table 1) (16–22). The interaction of sCD40L and glycoprotein IIb/IIIa receptor is important in thrombosis and thrombus stability. Glycoprotein IIb/IIIa inhibitors such as abciximab may provide benefit in this high-risk population (17), with the caveat being the increased levels of sCD40L and potential worsening of the proinflammatory state and increased mortality seen with GP IIb/IIIa inhibitor underdosing (23,24).

These observations support the premise that platelet activity is central to the proinflammatory and prothrombotic states in ACS. CD40L and sCD40L seem to link these processes and underscore the need to identify those at

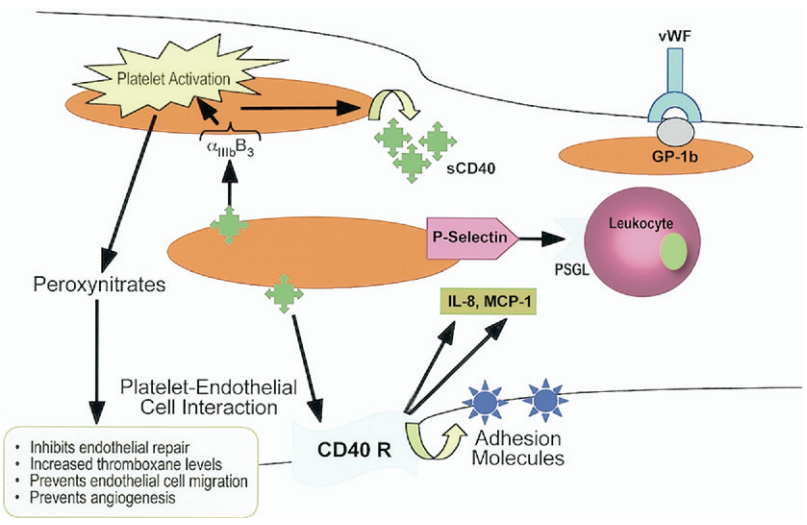

Figure 1 **Platelets: Key Mediators of Inflammation**

CD40R = CD40 receptor; GP-1b = glycoprotein 1b receptor; IL-8 = interleukin 8; MCP-1 = monocyte chemoattractant protein-1; PSGL = P-selectin glycoprotein ligand; sCD40 = soluble CD40; vWF = von Willebrand factor.

Table 1 Effect of Medical Therapy on CD40L

Study	Medical Intervention	CD40L vs. sCD40L	Clinical Outcome	Patient Type	Patients (n)	Comments
MIRACL (16)	Atorvastatin	sCD40L	Composite of adverse cardiovascular events	UA/NSTEMI	2,352	Atorvastatin resulted in a significant reduction in adverse cardiac events, notably in those with higher sCD40L
CAPTURE (17)	Abciximab	sCD40L	6 months composite of death or nonfatal MI	ACS	1,088 ACS 626 CP	Abciximab use was of more benefit in those with higher sCD40L levels
Schonbeck et al. (18)	HMG-CoA reductase inhibitors	sCD40L	Plasma levels of sCD40L	Primary prevention		Statin therapy led to a reduction in sCD40L
Sanguigni et al. (19)	Atorvastatin	sCD40L, platelet-bound CD40L	sCD40L/CD40L, F1 + 2 (marker of thrombin generation)	Primary prevention	30	Atorvastatin reduced sCD40L, platelet-bound CD40L and F1 + 2 suggesting anti-inflammatory and antithrombotic mechanisms at play
Varo et al. (20)	Troglitazone	sCD40L	sCD40L levels after 12 weeks of therapy	Type 2 DM	48	Troglitazone therapy resulted in a reduction in sCD40L over a 12-week period
Semb et al. (21)	Atorvastatin 80 mg/day vs. simvastatin 40 mg/day	sCD40L	sCD40L after 2 yrs of therapy	Familial hypercholesterolemia	110	Reduction in sCD40L seen with statin therapy was independent of effect on cholesterol
Furman et al. (22)	Abciximab vs. eptifibatide vs. control	sCD40L	sCD40L, leukocyte-platelet aggregates 18–24 h after PCI	ACS patients undergoing PCI	98	Reduction in sCD40L with abciximab and eptifibatide vs. control

ACS = acute coronary syndromes; CAD = coronary artery disease; CAPTURE = C7E3 Fab AntiPlatelet Therapy in Unstable REfractory angina; CP = chest pain; DM = diabetes mellitus; MI = myocardial infarction; MIRACL = Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering; NSTEMI = non–ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; UA = unstable angina.

higher risk, who may benefit from more aggressive or even more selective therapy.

Platelet-leukocyte interaction. The platelet serves as an intermediary between various cell types, most notably, the leukocytes. P-selectin, expressed on the surfaces of both endothelium and activated platelets, and platelet-leukocyte interactions that occur via P-selectin and its natural ligand P-selectin glycoprotein ligand-1 (PSGL-1) both appear to be important in thrombus generation.

Increased levels of soluble P-selectin have been shown to predict future cardiovascular events in apparently healthy women (25), to predict those patients with an ACS at high risk, and to potentially differentiate those patients with ACS versus stable angina (26). Despite these data, the utility of P-selectin as a marker of platelet activation in ACS remains uncertain. A more sensitive marker of thrombosis in patients with unstable coronary syndromes may be platelet-leukocyte aggregate levels (27).

Leukocytes

The inflammatory responses leading to the disruption of plaque and subsequent events in ACS is characterized by a varied cellular presence. The relationship between monocyte-derived macrophages and the pathogenesis of atherosclerotic coronary artery disease (CAD) has been well studied. In addition to macrophages, the importance of other leukocytes in these processes has become apparent.

Studies have suggested a relationship between leukocytosis and adverse cardiac events after acute MI (28) and ACS (29) has been demonstrated. The mechanisms by which leukocytosis may lead to worse outcomes may include proteolytic damage, leukocyte aggregation, microvascular obstruction, infarct expansion, electrical instability, and impaired revascularization among others (30).

Dichotomizing cell types involved in atherogenesis compared with the development of ACS is difficult. However, the neutrophil, a hallmark of acute inflammation, has been thought to be vital to acute plaque rupture with autopsy specimens of culprit lesions from acute MI patients demonstrating higher concentrations of activated neutrophils that in those without ACS (31). At the other end of the spectrum, infiltration of the atherosclerotic plaque with monocytes and eventual uptake of oxidized low-density lipoprotein (LDL) particles is thought to be central to formation of atherosclerotic plaque. Activated macrophages are believed to facilitate ongoing inflammation present within the plaque, and thus may be important in the initiation of ACS (Fig. 2).

Leukocyte secretory products and ACS. Several leukocyte secretory products, including myeloperoxidase, monocyte chemoattractant protein-1, various interleukins, matrix metalloproteinases, pregnancy-associated plasma protein A, leukotriene B_4, hepatocyte growth factor, and interferon gamma, have been associated with atherogenesis, atherothrombosis, ACS, and outcomes after ACS (Table 2)

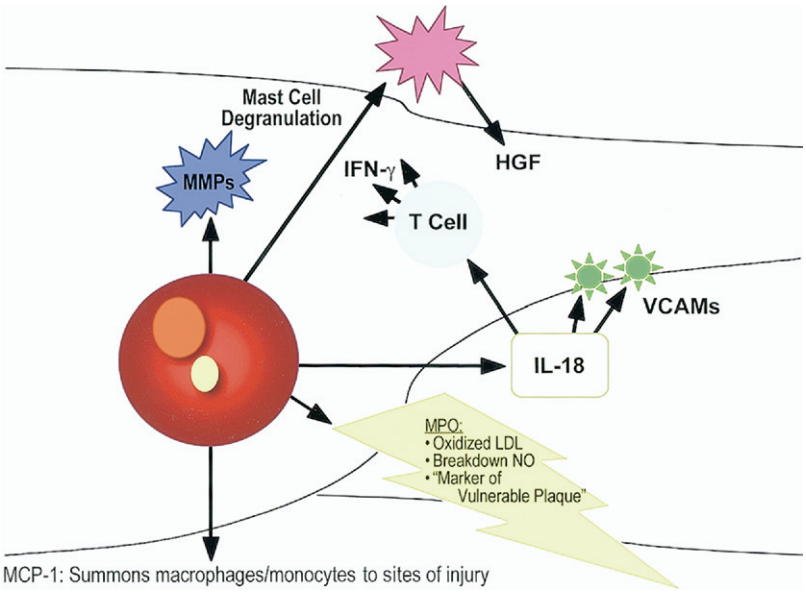

Figure 2 Leukocyte Secretory Products

HGF = hepatocyte growth factor; IFN-γ = interferon gamma; IL-18 = interleukin-18; LDL = low-density lipoprotein; MCP-1 = monocyte chemoattractant protein-1; MMPs = matrix metalloproteinases; MPO = myeloperoxidase; NO = nitric oxide; VCAMs = vascular cell adhesion molecule.

(32–51). For example, myeloperoxidase, a powerful oxidant released from both neutrophils and monocytes, has been demonstrated in patients with coronary artery disease (52), has been implicated in having a role in plaque destabilization (50), and has been associated with a worse prognosis in patients presenting with an ACS (51). Although the majority of these leukocyte secretory products link inflammation and atherothrombosis, their clinical applicability has

Table 2 Selected Leukocyte Secretory Products and Possible Involvement in ACS

Leukocyte Secretory Product	Contribution to Atherogenesis/ACS
Myeloperoxidase	Involved in low-density lipoprotein oxidation, nitric oxide breakdown, and endothelial homeostasis (32,33) Involved in plaque destabilization (50) Prognostic utility in those presenting with ACS (34,51) Involved in post-MI remodeling (35)
Monocyte chemoattractant protein-1	Essential for monocyte recruitment (36) Modulates inflammatory response in atherosclerosis, ACS, and postinfarct remodeling Higher levels associated with worse outcome post ACS (37)
Interleukin (IL)-18	Induces production of IFN-γ, MMP, and adhesion molecules Associated with "vulnerable plaque" morphology (38,39) Increased IL-18/IL-10 ratio correlates strongly with risk of adverse events in those with ACS (40)
Matrix metalloproteinases (MMPs)	MMP-2 and MMP-9 associated with plaque instability (41) Elevated levels of MMP-2 and MMP-9 seen in patients with ACS (42) MMP-9 was associated with increased risk of cardiovascular death in patients with ACS (43) Several isoforms, both cardiac and noncardiac, exist
Pregnancy-associated plasma protein-A (PAPP-A)	Activates insulin-like growth factor-1 Circulating PAPP-A significantly higher in patients with unstable coronary syndromes vs. those with stable angina (44) Elevated levels an independent predictor of 6-month major adverse cardiac events in patients with ACS (45) Several isoforms (both cardiac and non cardiac forms) exist
Hepatocyte growth factor	Related to thrombus generation in presence of mast cells (46) Significantly higher levels of hepatocyte growth factor demonstrated in patients with chest pain and evidence of acute thrombosis (47) Antiapoptotic Directly related to left ventricular function after MI (48)
Interferon gamma (IFN-γ)	Released from Th1 CD4+ cells Higher levels of Th1 CD4+ cells seen in patients with unstable angina vs. those with stable angina or controls (49) Activates monocytes/macrophages

ACS = acute coronary syndrome; MI = myocardial infarction.

not been adequately defined. Nevertheless, there exists encouraging data that emphasize the potential future utility of some, if not all of these products.

Progenitor Cells

Derived from bone marrow sources and peripheral mononuclear cells, circulating endothelial progenitor cells (EPCs) have been shown to possess several characteristics that may facilitate the use of these cells as novel markers of endothelial dysfunction as well as of ongoing tissue repair and/or regeneration. Characteristics such as their pluriopotency, ability to regenerate damaged endothelium, and the ability to "home" to damaged or ischemic tissue and contribute to neovascularization have elevated interest in these cells with novel prognostic and therapeutic goals in mind.

Endothelial progenitor cells represent a promising biomarker of endothelial dysfunction, one of the earliest stages of atherogenesis. In a study of patients with risk factors for CAD, reduced levels of EPCs correlated with higher degrees of endothelial dysfunction. Conversely, augmenting EPC volume through mechanisms, including transplantation, facilitates neovascularization and re-endothelialization and attenuates myocardial ischemia, supporting a role for EPCs in maintaining the homeostasis of the endothelial wall. Assessing for EPC levels may be more useful, given their correlation with established risk factors for CAD (53), their correlation with the presence of atherosclerosis (54), and with their prognostic ability in patients with established CAD (55). However, levels of circulating EPCs did not predict acute MI, suggesting a role for EPCs in atherosclerosis progression, but not in acute plaque rupture.

Although a natural, EPC-mediated repair mechanism that exists after myocardial injury has been demonstrated (56), this process occurs at a rate that precludes any meaningful functional recovery after MI. Experimental evidence has suggested that delivery of cytokine-expanded CD34+ EPCs via direct injection into the infarct border zone (57) may be able to augment the natural repair mechanisms and facilitate improvement in myocardial function. These observations highlight that a homing mechanism exists following myocardial injury, which, if harnessed, can contribute to myocardial repair facilitated, in part, by EPCs.

Although EPCs represent a promising biomarker of endothelial dysfunction, they also maintain an ability to participate in the repair process. In a healthy state, there is a role for progenitor cells in preservation of the endothelial wall. In states of endothelial dysfunction, reduced levels and functionality of EPCs and other circulating progenitor cells may impair these abilities and predispose to further injury. Manipulation of these progenitor cells may provide a therapeutic strategy for treating early stages of endothelial injury or preventing adverse remodeling after MI.

Adipocytes

Obesity has been identified as a risk factor for the development of the metabolic syndrome and subsequent cardiovascular disease. Specifically, visceral adipose tissue has been cited as the principal reservoir of adipocytes. Adipose tissue is a metabolically active organ that contains blood vessels and various active cell types. Acting through various endocrine and paracrine mechanisms, the relevance of adipose tissue to cardiovascular disease stems from its proinflammatory effects. Obesity, especially that which is associated with increased waist-to-hip ratio or increased visceral fat, leads to the upregulation of various intercellular adhesion molecules, P-selectin, C-reactive protein (CRP), interleukin-6, tumor necrosis factor-α, interleukin-18, tumor necrosis factor receptors, and plasminogen activator inhibitor-1, among others, are thought to result in a proinflammatory state that contributes to atherogenesis. Suppression of adiponectin, a protective adipokine, also appears to result from obesity.

Although adipocytes produce and secrete a variety of other factors, much is being learned about many of these factors and any relationship to coronary artery disease and ACS. Other examples include the transcription factor GATA2, resistin, CRP, serum amyloid A3, and leptin. Although these represent novel markers and may provide valuable insight with regard to atherosclerotic disease related to obesity, only CRP has been extensively studied in the context of coronary artery disease (Table 3) (58–80).

CRP. C-reactive protein is an acute phase reactant produced primarily by the liver in response to cytokines such as interleukin-6. It has gained attention not only as a marker of inflammation and cardiovascular risk but as an active participant in the process (81). C-reactive protein adds value beyond traditional cardiovascular risk factors such as LDL in predicting the risk of MI, stroke, need for revascularization, or death from cardiovascular causes (82). The significance of CRP is that it highlights the relationship between ongoing inflammation and future cardiac events. In those with unstable angina, discharge CRP levels predicted long-term risk of recurrent events (83).

Beyond prognostic value, evidence supports the direct involvement of CRP in the development of atherosclerotic plaque. C-reactive protein, identified in atherosclerotic plaque, has been shown to facilitate macrophage uptake of LDL particles (84) and to regulate both macrophage recruitment (85) and vascular adhesion molecule expression (86).

C-reactive protein has become an attractive target for medical therapy in coronary atherosclerosis (Table 4) (87–94). Therapeutic strategies in ACS have focused on modifiable risk factors, but none have focused on inflammation per se. It still remains to be seen what effect specifically targeting CRP will have upon hard clinical end points. A recent study in mice with experimental MI showed that CRP inhibition could markedly reduce infarct size (95). Although statin therapy has been touted for its ability to reduce levels of CRP (87–89,91) only now are studies under way examining

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Table 3 Adipocyte-Related Secretory Products: Relationship to Cardiovascular Disease

Secretory Product	Functional Role	Rationale for Involvement in Atherosclerosis	Clinical Evidence	Comments
Adiponectin	Important role in lipid and carbohydrate metabolism Modulates action of insulin	Prevents lipid uptake by macrophages + inhibits transformation into foam cells (58) Acts to increase TIMP-1 expression in macrophages in vitro (59) Affects expression of various cellular adhesion molecules by affecting NF-κB signaling (60,61)	Levels correlate inversely with future risk of CV events and CRP levels (62) Males with type II DM, 5-yr risk of fatal/nonfatal CAD inversely correlates with adiponectin (63) Nested case control data from HPFS, adiponectin inversely correlates with 6-yr risk of fatal/nonfatal CAD (64) Lower levels of adiponectin in those with established CAD vs. controls (65) Acute drop in adiponectin levels observed after AMI (66)	Likely to possess protective effects in relation to atherosclerotic disease. This needs to be further defined. However, it holds prognostic value in selected populations.
PAI-1	Functions to inhibit plasminogen activation and results in a balance favoring a prothrombotic state and may serve as a marker of impaired fibrinolysis	Prothrombotic PAI levels are often increased in vascular disease, including DVT and AMI Balance between PA/PAI-1 is affected in states such as obesity and DM	Small clinical study showing TnT and PAI-1 add prognostic information in the setting of ACS (67) In STEMI patients, acute rise in PAI-1 during first 24 hrs predicts 30-day mortality and risk for developing CHF (68)	May potentially serve as a link between inflammation and thrombosis. It may provide adipocytes a mechanism by which to modulate the thrombotic state.
VEGF	Angiogenic factor	Levels of visceral adipose tissue correlate with VEGF levels VEGF-induced angiogenesis may contribute to atherosclerotic lesions (69) In experimental models, VEGF has been shown to hasten the progression of atherosclerotic plaque (70,71) Suggestions have been made about neovascularization being a factor in plaque instability and rupture (72) VEGF appears to modulate effect of angiotensin II-mediated inflammation (73)	Treatment with statin therapy has been shown to reduce VEGF levels in those with established coronary atherosclerosis (74)	The significance of angiogenesis in the context of coronary artery disease remains controversial. There is phase I clinical trial data which show symptomatic benefit of VEGF via direct myocardial injection in those with refractory angina.
IL-6	Pro-inflammatory Increases production of CRP in the liver	Leads to production of CRP, a marker known to be associated with risk of future CV events Has been shown to decrease lipoprotein lipase activity	Elevated IL-6 levels in unstable angina patients predicted complicated in-hospital course (75)	By mediating production of CRP, in addition to affecting the production of vascular cellular adhesion molecules and affecting coagulation, IL-6 may modulate many of the events that promote atherosclerotic disease.
TNF-α	Has a role in obesity-related insulin resistance A cytokine involved in many proinflammatory disease states	In obesity, expression also increased on the surface of adipocytes Affects endothelial function by modulating expression of cellular adhesion molecules through NF-κB (76) Increases MMP activity (77) Inhibition of TNF-α in apoE knockout mice reduces disease progression (78)	Large-scale clinical data lacking. However, small studies have shown elevated levels of TNF-α in unstable coronary syndromes (79)	Although much experimental evidence suggests a potential mechanistic role for this adipocyte product in atherosclerosis and acute coronary syndromes, clinical data supporting its involvement are sparse.
Resistin	Has been shown to induce insulin resistance in rodents	Adipocyte product Levels correlate with other inflammatory markers such as IL-6	Plasma resistin levels predict coronary atherosclerosis in humans (based on coronary artery calcification as an index) (80)	Much more information is necessary to delineate the role for resistin in humans, especially in linking inflammation and atherosclerosis.

ACS = acute coronary syndrome; AMI = acute myocardial infarction; apo = apolipoprotein; CAD = coronary artery disease; CHF = congestive heart failure; CRP = C-reactive protein; CV = cardiovascular; DM = diabetes mellitus; DVT = deep venous thrombosis; HPFS = health professionals; IFN-γ = interferon gamma; IL-6 = interleukin-6; NF-κb = nuclear factor kappa B; PA = plasminogen activator; PAI-1 = plasminogen activator inhibitor-1; STEMI = ST-segment elevation myocardial infarction; TIMP-1 = Tissue inhibitor of metalloproteinase 1; TNF-α = tumor necrosis factor-alpha; TnT = troponin T; VEGF = vascular endothelial growth factor.

Table 4 Effect of Medical Therapy on CRP

Study	Patients (n)	Design	Outcome	Comments
PRINCE (87)	2,013 in primary prevention cohort 1,375 in secondary prevention cohort	Community-based, prospective double-blind, randomized	Pravastatin resulted in a statistically significant reduction in CRP both at 3 and 6 mos vs. placebo	Effect of statin therapy on CRP was independent of LDL level
CARE substudy (88)	391 patients from CARE who developed recurrent coronary events vs. controls	Nested case control study from CARE, a randomized, double-blind, placebo-controlled trial	Pravastatin resulted in a significant lowering of CRP; statin therapy resulted in a reduction in relative risk of further coronary events	Demonstrates anti-inflammatory effects of statin therapy; Possibility exists that pravastatin therapy may reduce risk of coronary events by controlling inflammation
PROVE IT–TIMI-22 substudy (89)	3,745 patients with ACS	Substudy of PROVE IT–TIMI-22, a randomized 2 × 2 factorial designed trial	Lower CRP after statin therapy in this group resulted in better clinical outcomes	Effect was noted to be independent of LDL cholesterol
REVERSAL (90)	657 patients randomized to 40 pravastatin or 80 atorvastatin	Double-blind, randomized active control multicenter trial	High-dose atorvastatin reduced CRP more than pravastatin; furthermore, high-dose therapy resulted in reduced atherosclerosis progression by IVUS	High-dose atorvastatin therapy reduced progression of atherosclerosis by IVUS, not a clinical end point trial
AFCAPS/TexCAPS substudy (91)	5,742 patients randomized to lovastatin vs. placebo	Substudy of double-blind, randomized, placebo-controlled study	Long term lovastatin therapy was effective in primary prevention in those with elevated CRP	Again, effect was independent of lipid profile changes
Haffner et al. (92)	357 patients randomized to rosiglitazone vs. placebo	Frozen blood samples analyzed from type II diabetics who participated in a 26-week randomized, double-blinded placebo-controlled trial using rosiglitazone	Rosiglitazone therapy reduced MMP and CRP levels, suggesting a potential beneficial effect upon cardiovascular risk	No hard clinical end points were examined
JUPITER (93,94)	Target population of 15,000 males > 55 and females > 65 years of age	Randomized, double-blind, placebo-controlled trial of rosuvastatin among those with elevated CRP and low LDL	Will evaluate primary end points of MI, stroke, UA, CV death, or revascularization	Hopes to answer whether statin therapy will reduce the risk of CV events in those with elevated CRP as a surrogate for inflammation

ACS = acute coronary syndrome; AFCAPS/TexCAPS = Air Force/Texas Coronary Atherosclerosis Prevention Study; CARE = Cholesterol and Recurrent Events Study; CRP = C-reactive protein; CV = cardiovascular; IVUS = intravascular ultrasound; JUPITER = Justification for the Use of Statins in Primary Prevention—Can CRP be Used to Target Statin Therapy in Primary Prevention; LDL = low-density lipoprotein; MI = myocardial infarction; MMP = matrix metalloproteinase; pop = population; PRINCE = Effect of Statin therapy on C-RP: Pravastatin Inflammation/CRP Evaluation; PROVE IT–TIMI-22 = Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22; REVERSAL = Reversal of Atherosclerosis w/ Aggressive Lipid Lowering; UA = unstable angina.

the effects of various therapeutic modalities upon CRP levels as a marker of clinical cardiovascular risk (93,94).

It is apparent that inflammatory status is a variable that needs to be strongly considered. Whether currently available therapy will reduce inflammation and improve clinical end points has yet to be determined. Nevertheless, CRP provides valuable insight regarding the link between inflammation, CAD, and ACS.

Genetic Risk, Molecular Risk Factors, and Clinical Medicine

Genetic predisposition toward the development of MI is a concept that is only bluntly defined by traditional risk factors such as hypertension or hyperlipidemia. This common complex trait with extensive gene-environment and gene-gene interactions is in the early phase of being genomically unraveled. Complexity of the process is reflected in the number of single nucleotide polymorphisms. For example, the inhibition of the recently identified 5-lipoxygenase activating pathway with a 5-lipoxygenase activating protein pathway (FLAP) blocker in patients with a gain-of-function FLAP or leukotriene A4 haplotype has been shown to reduce the degree of inflammation as measured by various proinflammatory biomarkers, including CRP and leukotriene B4. Similarly, specific variants of PCSK-9 and USF-1, which affect lipoprotein handling, have been shown to provide marked protection from ACS events (96–99). This is a promising example of how such specific genomic information could facilitate individualized prevention.

With high-throughput genotyping of >500,000 key marker single nucleotide polymorphisms, the ability to identify the susceptibility factors for ACS such as FLAP or leukotriene A4 is greatly enhanced. High-throughput sequencing tools and microarrays will allow for examination and comparison of thousands of genes at once. The potential exists for developing profiles of risk based on genetic information. Ginsburg et al. (100) discuss the value of personalized cardiovascular medicine using not only large-scale genetic profiles but also gene products in revolutionizing the scope of clinical practice. Improved diagnostic sensitivity and refined prognostic value in combination with a tailored therapeutic approach would be the proposed outcome.

More and more genes and gene products are being considered as being valuable in providing information about patients at risk for ACS. As more candidates are introduced

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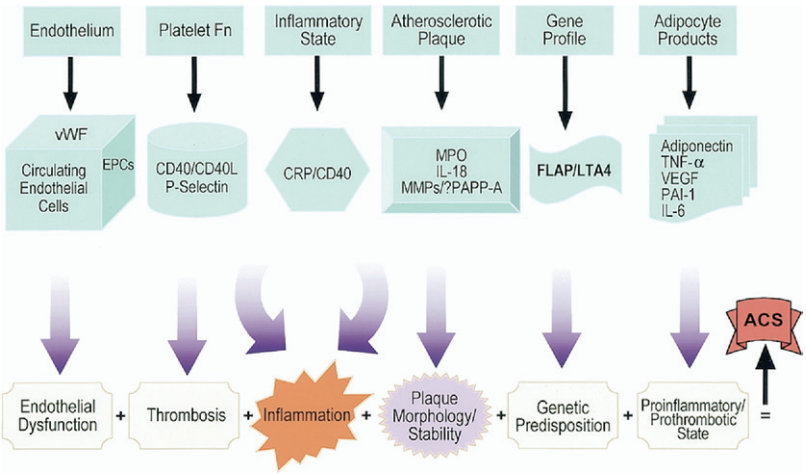

Figure 3 **A Model of Risk Stratification Based on a Representative Panel of Molecular and Genetic Factors**

ACS = acute coronary syndrome; CRP = C-reactive protein; EPC = endothelial progenitor cell; FLAP = 5-lipoxygenase activating protein pathway; fn = platelet function; IL = interleukin; LTA4 = leukotriene A4 pathway; MMPs = matrix metalloproteinases; MPO = myeloperoxidase; PAI-1 = plasminogen activator inhibitor; PAPP-A = pregnancy-associated plasma protein A; sCD40L = soluble CD40 ligand; TNF-α = tumor necrosis factor alpha; VEGF = vascular endothelial growth factor; vWF = Von Willebrand factor.

through proteomics and metabolomics, their pragmatic utility must be questioned in dedicated clinical trials. A standardized panel of markers used to assess inflammation, plaque vulnerability, and other features may become part of clinical practice, but the selection of which markers to use remains undecided, especially as the selection pool grows in size and complexity. Most of the markers that have been discussed have not yet been examined concurrently in large-scale clinical epidemiologic studies. Transitioning these markers directly into clinical practice without sufficient data stands only to create confusion. Furthermore, of the markers that hold promise in clinical medicine, there is still much to be learned about specific assays, measurement characteristics, and more precise pathophysiologic definitions. sCD40L is an excellent example of this as sample processing and temperature were found to affect measurements (101)—despite our current knowledge, our understanding still remains limited.

Although the discovery of such markers and subsequent studies proving association with ACS will likely continue to take place at an accelerated pace, the rate-limiting step should involve a rigorous process of systematically evaluating these markers prior to transitioning into clinical practice. This would include reproducibility in large populations, a scrutiny of assay methods, cost effectiveness appraisals, an assessment of practicality, and determination of whether value is added beyond current methods of risk stratification.

Conclusions. It is apparent that a myriad of cellular and molecular mediators involved in the proinflammatory and prothrombotic phases of atherosclerosis and ACS exist. What determines any individual's clinical manifestations may reflect the interplay of inflammatory components, environmental factors, and genetic susceptibility. Although our understanding of the inflammatory processes is only now expanding, we are just scratching the surface with regard to genetic susceptibility in acute coronary syndromes.

What remains wholly apparent, however, is the complexity of this disease process. It is no wonder, then, that many elements have been individually identified, characterized, and studied in the clinical setting in an effort to understand at least one potential pathway. Although no one entity has ever been found to be the holy grail of the ACS, the understanding of the complex interplay between these components seems to be most important.

In time, risk assessment may take the form of an evaluation of multiple molecular factors using a comprehensive pan-arterial analysis of carefully selected candidate genes and molecules that reflect the variety of cellular and molecular components actively involved in the pathogenesis of clinically apparent disease (Fig. 3).

Although using such a panel for disease risk stratification remains an objective, monitoring disease activity and pursuing individualized disease prevention are possible outgrowths of this work. In those with known disease, monitoring for evidence of ongoing inflammation, endothelial dysfunction, or platelet activation may help to identify those at higher risk requiring more intensive or more specific therapy to avert future events. Ultimately, real promise may turn out for primary prevention whereby the use of a panel of molecular markers and candidate genes may identify a particular segment of the population at risk for clinically significant CAD, otherwise undetectable. Screening for early evidence of endothelial dysfunction, up-regulation of inflammation, thrombosis, or genetic susceptibility will likely provide a new more precise assessment of risk for future cardiovascular disease beyond traditional clinical risk factors.

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REFERENCES

1. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
2. Buffon A, Biasucci LM, Liuzzo G, D'Onofrio G, Crea F, Maseri A. Widespread coronary inflammation in unstable angina. N Engl J Med 2002;347:5–12.
3. Topol EJ. Simon Dack lecture. The genomic basis of myocardial infarction. J Am Coll Cardiol 2005;46:1456–65.
4. Morrow DA, Braunwald E. Future of biomarkers in acute coronary syndromes: moving toward a multimarker strategy. Circulation 2003; 108:250–2.
5. Mutin M, Canavy I, Blann A, Bory M, Sampol J, Dignat-George F. Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. Blood 1999;93:2951–8.
6. Montalescot G, Collet JP, Choussat R, Ankri A, Thomas D. A rise of troponin and/or von Willebrand factor over the first 48 h is associated with a poorer 1-year outcome in unstable angina patients. Int J Cardiol 2000;72:293–4.
7. Andre P, Denis CV, Ware J, et al. Platelets adhere to and translocate on von Willebrand factor presented by endothelium in stimulated veins. Blood 2000;96:3322–8.
8. Eto K, Isshiki T, Yamamoto H, et al. AJvW-2, an anti-vWF monoclonal antibody, inhibits enhanced platelet aggregation induced by high shear stress in platelet-rich plasma from patients with acute coronary syndromes. Arterioscler Thromb Vasc Biol 1999;19:877–82.
9. Ray KK, Morrow DA, Gibson CM, Murphy S, Antman EM, Braunwald E. Predictors of the rise in vWF after ST elevation myocardial infarction: implications for treatment strategies and clinical outcome: an ENTIRE-TIMI 23 substudy. Eur Heart J 2005;26: 440–6.
10. Montalescot G, Philippe F, Ankri A, et al. Early increase of von Willebrand factor predicts adverse outcome in unstable coronary artery disease: beneficial effects of enoxaparin. French Investigators of the ESSENCE trial. Circulation 1998;98:294–9.
11. Wagner AH, Guldenzoph B, Lienenluke B, Hecker M. CD154/CD40-mediated expression of CD154 in endothelial cells: consequences for endothelial cell-monocyte interaction. Arterioscler Thromb Vasc Biol 2004;24:715–20.
12. Andre P, Prasad KS, Denis CV, et al. CD40L stabilizes arterial thrombi by a beta3 integrin-dependent mechanism. Nat Med 2002; 8:247–52.
13. Furman MI, Krueger LA, Linden MD, Barnard MR, Frelinger AL 3rd, Michelson AD. Release of soluble CD40L from platelets is regulated by glycoprotein IIb/IIIa and actin polymerization. J Am Coll Cardiol 2004;43:2319–25.
14. Urbich C, Dernbach E, Aicher A, Zeiher AM, Dimmeler S. CD40 ligand inhibits endothelial cell migration by increasing production of endothelial reactive oxygen species. Circulation 2002;106:981–6.
15. Varo N, de Lemos JA, Libby P, et al. Soluble CD40L: risk prediction after acute coronary syndromes. Circulation 2003;108:1049–52.
16. Kinlay S, Schwartz GG, Olsson AG, et al. Effect of atorvastatin on risk of recurrent cardiovascular events after an acute coronary syndrome associated with high soluble CD40 ligand in the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) Study. Circulation 2004;110:386–91.
17. Heeschen C, Dimmeler S, Hamm CW, et al. Soluble CD40 ligand in acute coronary syndromes. N Engl J Med 2003;348:1104–11.
18. Schonbeck U, Gerdes N, Varo N, et al. Oxidized low-density lipoprotein augments and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors limit CD40 and CD40L expression in human vascular cells. Circulation 2002;106:2888–93.
19. Sanguigni V, Pignatelli P, Lenti L, et al. Short-term treatment with atorvastatin reduces platelet CD40 ligand and thrombin generation in hypercholesterolemic patients. Circulation 2005;111:412–9.
20. Varo N, Vicent D, Libby P, et al. Elevated plasma levels of the atherogenic mediator soluble CD40 ligand in diabetic patients: a novel target of thiazolidinediones. Circulation 2003;107:2664–9.
21. Semb AG, van Wissen S, Ueland T, et al. Raised serum levels of soluble CD40 ligand in patients with familial hypercholesterolemia: downregulatory effect of statin therapy. J Am Coll Cardiol 2003;41: 275–9.
22. Furman MI, Krueger LA, Linden MD, et al. GPIIb-IIIa antagonists reduce thromboinflammatory processes in patients with acute coronary syndromes undergoing percutaneous coronary intervention. J Thromb Haemost 2005;3:312–20.
23. Nannizzi-Alaimo L, Alves VL, Phillips DR. Inhibitory effects of glycoprotein IIb/IIIa antagonists and aspirin on the release of soluble CD40 ligand during platelet stimulation. Circulation 2003;107: 1123–8.
24. Quinn MJ, Plow EF, Topol EJ. Platelet glycoprotein IIb/IIIa inhibitors: recognition of a two-edged sword? Circulation 2002;106: 379–85.
25. Ridker PM, Buring JE, Rifai N. Soluble P-selectin and the risk of future cardiovascular events. Circulation 2001;103:491–5.
26. Guray U, Erbay AR, Guray Y, et al. Levels of soluble adhesion molecules in various clinical presentations of coronary atherosclerosis. Int J Cardiol 2004;96:235–40.
27. Freedman JE, Loscalzo J. Platelet-monocyte aggregates: bridging thrombosis and inflammation. Circulation 2002;105:2130–2.
28. Menon V, Lessard D, Yarzebski J, Furman MI, Gore JM, Goldberg RJ. Leukocytosis and adverse hospital outcomes after acute myocardial infarction. Am J Cardiol 2003;92:368–72.
29. Sabatine MS, Morrow DA, Cannon CP, et al. Relationship between baseline white blood cell count and degree of coronary artery disease and mortality in patients with acute coronary syndromes: a TACTICS-TIMI 18 (Treat Angina with Aggrastat and determine Cost of Therapy with an Invasive or Conservative Strategy-Thrombolysis in Myocardial Infarction 18 trial) substudy. J Am Coll Cardiol 2002;40:1761–8.
30. Madjid M, Awan I, Willerson JT, Casscells SW. Leukocyte count and coronary heart disease: implications for risk assessment. J Am Coll Cardiol 2004;44:1945–56.
31. Naruko T, Ueda M, Haze K, et al. Neutrophil infiltration of culprit lesions in acute coronary syndromes. Circulation 2002;106:2894–900.
32. Podrez EA, Febbraio M, Sheibani N, et al. Macrophage scavenger receptor CD36 is the major receptor for LDL modified by monocyte-generated reactive nitrogen species. J Clin Invest 2000;105:1095–108.
33. Abu-Soud HM, Hazen SL. Nitric oxide modulates the catalytic activity of myeloperoxidase. J Biol Chem 2000;275:5425–30.
34. Brennan ML, Penn MS, Van Lente F, et al. Prognostic value of myeloperoxidase in patients with chest pain. N Engl J Med 2003; 349:1595–604.
35. Askari AT, Brennan ML, Zhou X, et al. Myeloperoxidase and plasminogen activator inhibitor 1 play a central role in ventricular remodeling after myocardial infarction. J Exp Med 2003;197:615–24.
36. Dewald O, Zymek P, Winkelmann K, et al. CCL2/monocyte chemoattractant protein-1 regulates inflammatory responses critical to healing myocardial infarcts. Circ Res 2005;96:881–9.
37. de Lemos JA, Morrow DA, Sabatine MS, et al. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003;107:690–5.
38. de Nooijer R, von der Thusen JH, Verkleij CJ, et al. Overexpression of IL-18 decreases intimal collagen content and promotes a vulnerable plaque phenotype in apolipoprotein-E-deficient mice. Arterioscler Thromb Vasc Biol 2004;24:2313–9.
39. Mallat Z, Corbaz A, Scoazec A, et al. Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. Circ Res 2001;89:E41–5.
40. Chalikias GK, Tziakas DN, Kaski JC, et al. Interleukin-18: interleukin-10 ratio and in-hospital adverse events in patients with acute coronary syndrome. Atherosclerosis 2005;182:135–43.
41. Shah PK, Falk E, Badimon JJ, et al. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. Circulation 1995;92: 1565–9.

42. Kai H, Ikeda H, Yasukawa H, et al. Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. J Am Coll Cardiol 1998;32:368–72.
43. Blankenberg S, Rupprecht HJ, Poirier O, et al. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation 2003;107: 1579–85.
44. Bayes-Genis A, Conover CA, Overgaard MT, et al. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. N Engl J Med 2001;345:1022–9.
45. Lund J, Qin QP, Ilva T, et al. Circulating pregnancy-associated plasma protein a predicts outcome in patients with acute coronary syndrome but no troponin I elevation. Circulation 2003;108: 1924–6.
46. Kinoshita M, Miyamoto T, Ohashi N, Sasayama S, Matsumori A. Thrombosis increases circulatory hepatocyte growth factor by degranulation of mast cells. Circulation 2002;106:3133–8.
47. Hata N, Matsumori A, Yokoyama S, et al. Hepatocyte growth factor and cardiovascular thrombosis in patients admitted to the intensive care unit. Circ J 2004;68:645–9.
48. Yasuda S, Goto Y, Baba T, et al. Enhanced secretion of cardiac hepatocyte growth factor from an infarct region is associated with less severe ventricular enlargement and improved cardiac function. J Am Coll Cardiol 2000;36:115–21.
49. Zhou X, Nicoletti A, Elhage R, Hansson GK. Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. Circulation 2000;102:2919–22.
50. Fu X, Kassim SY, Parks WC, Heinecke JW. Hypochlorous acid oxygenates the cysteine switch domain of pro-matrilysin (MMP-7). A mechanism for matrix metalloproteinase activation and atherosclerotic plaque rupture by myeloperoxidase. J Biol Chem 2001;276: 41279–87.
51. Baldus S, Heeschen C, Meinertz T, et al. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation 2003;108:1440–5.
52. Zhang R, Brennan ML, Fu X, et al. Association between myeloperoxidase levels and risk of coronary artery disease. JAMA 2001;286: 2136–42.
53. Vasa M, Fichtlscherer S, Aicher A, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res 2001;89:E1–7.
54. Fadini GP, Miorin M, Facco M, et al. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. J Am Coll Cardiol 2005;45:1449–57.
55. Werner N, Kosiol S, Schiegl T, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005; 353:999–1007.
56. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001;107:1395–402.
57. Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430–6.
58. Ouchi N, Kihara S, Arita Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation 2001;103:1057–63.
59. Kumada M, Kihara S, Ouchi N, et al. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation 2004; 109:2046–9.
60. Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999;100:2473–6.
61. Ouchi N, Kihara S, Arita Y, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000;102:1296–301.
62. Ouchi N, Kihara S, Funahashi T, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003;107:671–4.
63. Schulze MB, Shai I, Rimm EB, Li T, Rifai N, Hu FB. Adiponectin and future coronary heart disease events among men with type 2 diabetes. Diabetes 2005;54:534–9.
64. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004;291:1730–7.
65. Kumada M, Kihara S, Sumitsuji S, et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003;23:85–9.
66. Kojima S, Funahashi T, Sakamoto T, et al. The variation of plasma concentrations of a novel, adipocyte derived protein, adiponectin, in patients with acute myocardial infarction. Heart 2003;89:667.
67. Sinkovic A, Pogacar V. Risk stratification in patients with unstable angina and/or non-ST-elevation myocardial infarction by Troponin T and plasminogen-activator-inhibitor-1 (PAI-1). Thromb Res 2004;114:251–7.
68. Collet JP, Montalescot G, Vicaut E, et al. Acute release of plasminogen activator inhibitor-1 in ST-segment elevation myocardial infarction predicts mortality. Circulation 2003;108:391–4.
69. Inoue M, Itoh H, Ueda M, et al. Vascular endothelial growth factor (VEGF) expression in human coronary atherosclerotic lesions: possible pathophysiological significance of VEGF in progression of atherosclerosis. Circulation 1998;98:2108–16.
70. Celletti FL, Waugh JM, Amabile PG, Brendolan A, Hilfiker PR, Dake MD. Vascular endothelial growth factor enhances atherosclerotic plaque progression. Nat Med 2001;7:425–9.
71. Celletti FL, Hilfiker PR, Ghafouri P, Dake MD. Effect of human recombinant vascular endothelial growth factor165 on progression of atherosclerotic plaque. J Am Coll Cardiol 2001;37:2126–30.
72. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. Circulation 1999;99:1726–32.
73. Zhao Q, Ishibashi M, Hiasa K, Tan C, Takeshita A, Egashira K. Essential role of vascular endothelial growth factor in angiotensin II-induced vascular inflammation and remodeling. Hypertension 2004;44:264–70.
74. Alber HF, Dulak J, Frick M, et al. Atorvastatin decreases vascular endothelial growth factor in patients with coronary artery disease. J Am Coll Cardiol 2002;39:1951–5.
75. Biasucci LM, Liuzzo G, Fantuzzi G, et al. Increasing levels of interleukin (IL)-1Ra and IL-6 during the first 2 days of hospitalization in unstable angina are associated with increased risk of in-hospital coronary events. Circulation 1999;99:2079–84.
76. Bhagat K, Vallance P. Inflammatory cytokines impair endothelium-dependent dilatation in human veins in vivo. Circulation 1997;96: 3042–7.
77. Rajavashisth TB, Xu XP, Jovinge S, et al. Membrane type 1 matrix metalloproteinase expression in human atherosclerotic plaques: evidence for activation by proinflammatory mediators. Circulation 1999;99:3103–9.
78. Branen L, Hovgaard L, Nitulescu M, Bengtsson E, Nilsson J, Jovinge S. Inhibition of tumor necrosis factor-alpha reduces atherosclerosis in apolipoprotein E knockout mice. Arterioscler Thromb Vasc Biol 2004;24:2137–42.
79. Waehre T, Halvorsen B, Damas JK, et al. Inflammatory imbalance between IL-10 and TNFalpha in unstable angina potential plaque stabilizing effects of IL-10. Eur J Clin Invest 2002;32:803–10.
80. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation 2005;111:932–9.
81. Bhatt DL, Topol EJ. Need to test the arterial inflammation hypothesis. Circulation 2002;106:136–40.
82. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002;347: 1557–65.
83. Biasucci LM, Liuzzo G, Grillo RL, et al. Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. Circulation 1999;99:855–60.
84. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. Circulation 2001;103:1194–7.
85. Torzewski M, Rist C, Mortensen RF, et al. C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. Arterioscler Thromb Vasc Biol 2000;20:2094–9.

86. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation 2000;102: 2165–8.
87. Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/ CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA 2001;286:64–70.
88. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation 1999;100:230–5.
89. Ridker PM, Cannon CP, Morrow D, et al. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20–8.
90. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. JAMA 2004; 291:1071–80.
91. Ridker PM, Rifai N, Clearfield M, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001;344:1959–65.
92. Haffner SM, Greenberg AS, Weston WM, Chen H, Williams K, Freed MI. Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. Circulation 2002;106:679–84.
93. Ridker PM. High-sensitivity C-reactive protein, inflammation, and cardiovascular risk: from concept to clinical practice to clinical benefit. Am Heart J 2004;148:S19–26.
94. Ridker PM. Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: rationale and design of the JUPITER trial. Circulation 2003;108:2292–7.
95. Pepys MB, Hirschfield GM, Tennent GA, et al. Targeting C-reactive protein for the treatment of cardiovascular disease. Nature 2006;440:1217–21.
96. Helgadottir A, Manolescu A, Thorleifsson G, et al. The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. Nat Genet 2004;36:233–9.
97. Hakonarson H, Thorvaldsson S, Helgadottir A, et al. Effects of a 5-lipoxygenase-activating protein inhibitor on biomarkers associated with risk of myocardial infarction: a randomized trial. JAMA 2005;293:2245–56.
98. Cohen JC, Boerwinkle E, Mosley TH Jr., Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 2006;354:1264–72.
99. Komulainen K, Alanne M, Auro K, et al. Risk alleles of USF1 gene predict cardiovascular disease of women in two prospective studies. PLoS Genet 2006;2:e69.
100. Ginsburg GS, Donahue MP, Newby LK. Prospects for personalized cardiovascular medicine: the impact of genomics. J Am Coll Cardiol 2005;46:1615–27.
101. Ahn ER, Lander G, Jy W, et al. Differences of soluble CD40L in sera and plasma: implications on CD40L assay as a marker of thrombotic risk. Thromb Res 2004;114:143–8.